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Antimicrobial Activity of a Peptide from the Flowers of Rosa indica (L.)

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Abstract: ***Extraction, isolation and purification of an antimicrobial peptide was carried out from the flowers of Rosa indica and its antimicrobial activity was monitored against Escherichia coli and Staphylococcus aureus. The results showed that Rosa indica flowers purified extract has antibacterial activity against Escherichia coli and Staphylococcus aureus. Hence providing protection to the flowers against pathogen invasion.***

Keywords: *Rosa indica*; Peptide; Antimirobial; Purification

1. Introduction

Microbes produce various types of infections in living organisms. But the nature has provided the living organisms the capability to produce a large number of compounds to defend themselves against various infections. Antimicrobial peptides are one of these compounds.The antimicrobial peptides are ubiquitous among all eukaryotes including mammals,insect and plants[1].They act as first line of defense against pathogen in vertebrates[2].The plant Kingdome also produces these antimicrobial peptides. In plants the defensins are well known antimicrobial peptides. They were first isolated from wheat and barley.They have structural and functional similarities with insect and mammalian defensins[3].They are known for their antimicrobial activity against various pathogens especially bacterial species that are phytopathogenic [4]-[7]. In this paper we describe the isolation and biological properties of a flower derived defensin from *Rosaindica*.

2. Materials and Methods

2.1Biological materials

The plants were purchased from the nursery of Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India. The bacterial strains against which the antimicrobial activity was monitored were *E.coli*(MTCC 1687) and *S.aureus*(MTCC 7443).The bacterial strains were provided by Microbial Type Culture Collection and Gene Bank (MTCC),Institute of Microbial Technology,IMTECH,Chandigarh,India and the monitoring of the antimicrobial activity of the *Rosa indica* was carried out

2.2 Extraction of floral defensins

Defensins were extracted from flowers of *Rosa indica* by using a modification of the procedure for extraction of thionins from barley (*Hordeum vulgare*) flour [8] and further modification of the procedure used for isolation of floral defensins from ornamental tobacco and petunia [9]. Whole of *Rosa indica* flowers up to petal colouration stage of flower development were grounded to a fine powder with liquid nitrogen by using a mortar and pestle and were further processed in a homogenizer in 50mM sulphuric acid. After stirring for 1 hr at 4°C, the insoluble material was removed by filteration through Whatman No. 4 filter paper, followed by centrifugation (12,000 rpm,15 min, 4°C).The slurry was adjusted to a pH 7.8 by slow addition of 10 M NaOH and was stirred for 1 h at 4°C before removal of precipitated material by centrifugation (12,000 rpm,15 min, 4°C). Solid ammonium sulphate 80 % (w/v) saturation was added and the mixture was stirred for 3 to 4 h at 4°C to precipitate the defensin protein. The precipitate was collected and was dissolved in 50 ml of gel filteration buffer (150mM KCl and 10 mMTris-HCl,pH 8.0) before heating at 90°C for 10 min. The supernatant was loaded onto a glass column containing Sephadex G-50 (Sigma) media and gel chromatography was carried out.

Fractions with highest antibacterial activity were selected for each peak and were purified by RP-HPLC (Waters) on a C18silica column using a pump (model 515,Waters)and photodiode array detector (UV;Waters). Samples were eluted with linear gradient of 0.1%(v/v) trifluoroacetic acid to 60% (v/v) acetonitrile for 20 min at a flow rate of 2ml/min. The absorbance was measured till 700nm.

2.3 Antimicrobial screening

The purified protein extracts of *Rosa indica* were screened against two bacterial strains. The test organisms were *Escherichia coli* (MTCC 1687) and Staphylococcus aureus (MTCC 7443).

2.4 Preparation of inoculum

Slopes of nutrient agar were prepared in which stock cultures were maintained at 4°C.The active cultures of

bacteria were prepared for experiments by transferring a loopful of bacteria from stock cultures to test tubes of Mueller-Hinton Broth (MHB). A turbidity standard for inoculum preparation was used, a BaSO4turbidity standard, equivalent to a 0.5 McFarland standard.

2.5 Antimicrobial Susceptibility test

Kirby-Bauer disc diffusion method was used to screen the antimicrobial activity of the peptide extracts. In this test the antimicrobial activity was screened by using Mueller Hinton Agar (MHA) as media, obtained from (Himedia, Mumbai).The MHA plates were prepared by pouring 15 ml of molten media into the sterile petri plates. The plates were allowed to solidify for 5 minutes and inoculum suspension was swabbed uniformly, thesuspension was allowed to dry for 5 minutes. Sterile discs (6mm) were loaded with 50 µl of peptide extract. The loaded disc was placed on the surface of media and the compound was allowed to diffuse for 5 minutes and then the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones were formed around the disc, they were measured with a transparent ruler (Himedia) in millimetre.

3. Results and Discussions

The protein was extracted from flowers of *Rosa indica* in 50mM sulphuric acid and purified using ammonium sulphate precipitation, heat treatment and gel filtration. Proteins in the fractions were resolved further by Reverse Phase HPLC(RP-HPLC). The highest peak was identified as a floral defensin(figures 1 and 2).The highest peak was observed at a retention time of 2.476 minutes.

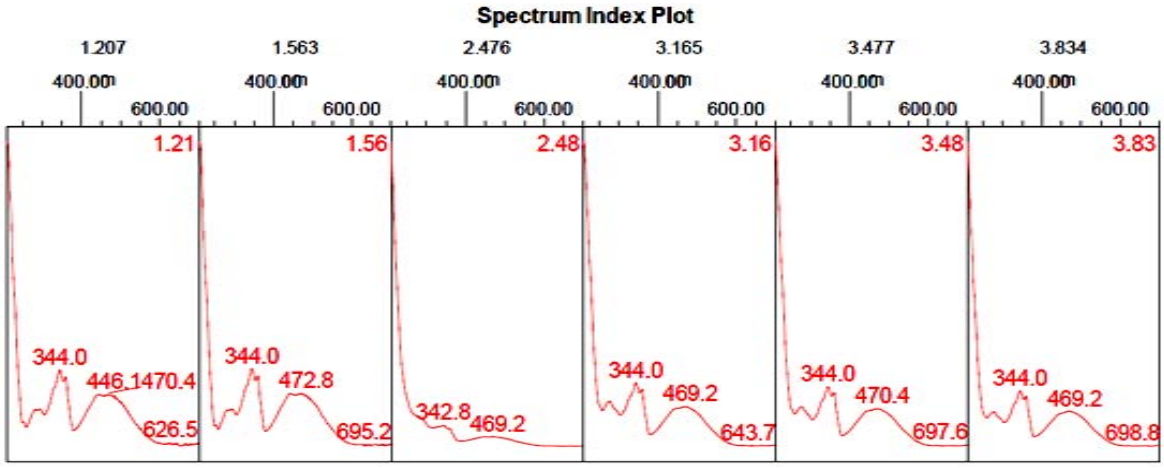

Figure 1: Spectrum Index plot of *Rosa indica* extract.

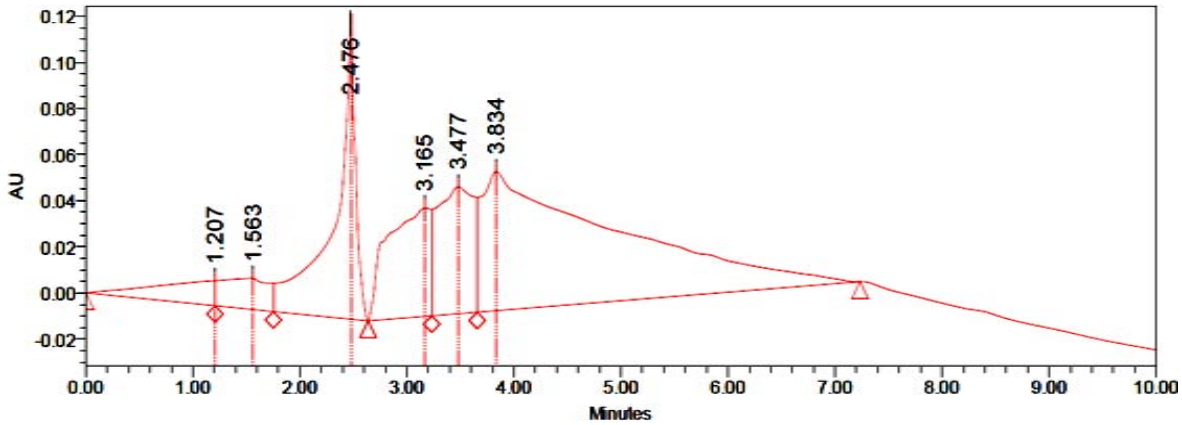

Figure 2: Retention time in minutes of *Rosa indica* extract.

3.1 Antimicrobial activity of the purified extracts

The purified peptide extracts of *Rosa indica* showed a zone of clearance against both the bacteria *E.coli* and *S.aureus*. A zone of clearance of 11 mm was observed against *E.coli*and a zone of clearance of 12 mm was observed against *S.aureus*(figures3, 4 and 5) respectively.

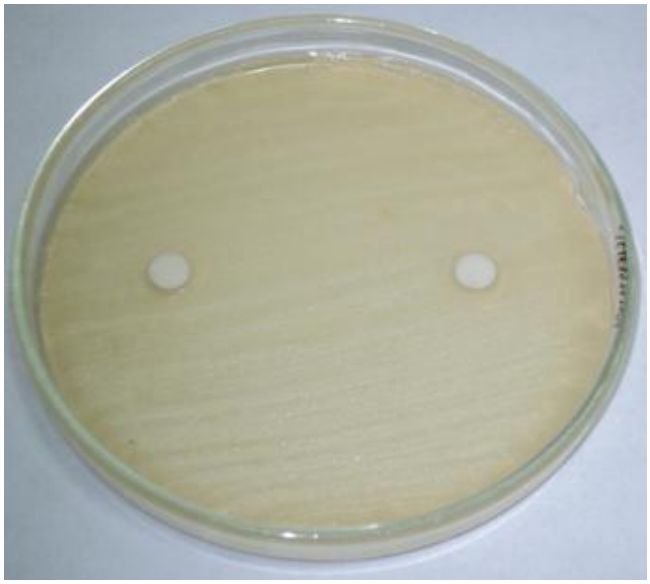

Figure 3: Antimicrobial activity of *Rosa indica* extracts against *E.coli*.

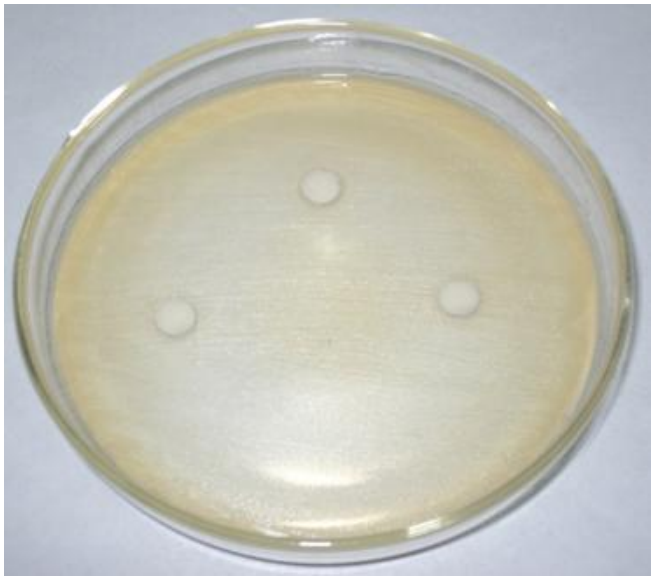

Figure 4: Antimicrobial activity of *Rosa indica* extracts against *E.coli*.

Figure 5: Antimicrobial activity of *Rosa indica* extracts against *S.aureus.*

This observation has lead towards the isolation of an antimicrobial peptide and the detection of the antimicrobial activity of this compound from *Rosa indica* flowers. The extracted compound shows antimicrobial activity. This is a promising compound which can have various applications in agriculture as well as medicine. Plants prove to be a source of antimicrobial compounds of peptide nature. Further investigation is required for screening of this compound in other parts of the plant like seeds and leaves.

4. Conclusion

This study has produced some convincing results as an antimicrobial peptide has been isolated from flowers of *Rosa indica* which is showing antimicrobial activity. This compound has been well detected by Reverse Phase HPLC (RP-HPLC) technique and a marked antibacterialactivity was observed against bacteria like *E.coli* and *S.aureus*.

5. Acknowledgements

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